

EXHIBIT A

DOCKET NO.: J&J-1673
Application No.: 09/486,757



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Toni M. Kutchan, Meinhart H. Zenk,
David G. Atkins, Anthony J. Fist

Confirmation No.: 4581

Application No.: 09/486,757

Group Art Unit: 1635

Filing Date: July 3, 2000

Examiner: Zara, Jane J.

For: Cytochrome P450 Reductases From Poppy Plants

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

**DECLARATION OF ANTHONY FIST
PURSUANT TO 37 C.F.R. §1.132**

I, Anthony John Fist, being duly warned that willful false statements and the like may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.
2. I am currently Manager of Agricultural Research and Development at Tasmanian Alkaloids Pty Ltd. In this role, I have been managing research and development work in many aspects of poppy production since 1991.
3. I graduated from the University of Queensland in 1987 with a Ph.D. degree in the field of agriculture. A copy of my *curriculum vitae* is attached hereto as Exhibit B.

4. The present invention provides, *inter alia*, methods of producing genetically modified poppy plants having different alkaloid content when compared to non-genetically modified poppy plants, as well as straw and alkaloids isolated from such plants.

5. I am a named inventor on the above-referenced patent application. I have read and I am familiar with the contents of the patent application. In addition, I have read the Office Action dated September 9, 2002, received in the present case. It is my understanding that the Examiner does not believe that overexpression of the cytochrome P-450 reductase gene will result in alteration in the production of alkaloids in poppy plants.

6. In order to demonstrate that over-expression of cytochrome P-450 reductase in a poppy plant does result in increased production of alkaloid content, I will describe the results of a greenhouse trial and field trial of genetically modified poppy plants containing a cytochrome P-450 reductase transgene. The trials were conducted in Tasmania, Australia. The trials were conducted in my department and were overseen by me.

7. A greenhouse trial was sown in mid-October 2002 and harvested in late February 2003. Twenty-one genetically modified poppy lines having the cytochrome P-450 reductase ("CPR") transgene from the California poppy plant were assessed against parent control lines for alkaloid accumulation. Five poppy plants were established per pot for the majority of treatments. Two pots were used for each treatment, and the trial was replicated four times. Transgenic lines that contained the CPR transgene originated from a CO48ib parent background. Of 21 lines tested, 3 lines (eCPR-8951, 89254 and 89266) had significant increases in total alkaloids compared to the parent controls. Transgenic line eCPR-89266 had significant increases in both thebaine and codeine. Transgenic line eCPR-8951 had significant increases in thebaine and more moderate increases in morphine. Transgenic line eCPR-89254 had a significant increase in thebaine. The results of the greenhouse trial demonstrate that alkaloid content was affected by introduction of the P-450 reductase transgene. Figure 5 is attached as Exhibit C and provides a comparison of greenhouse grown CO48ib transgenic lines and parent controls.

8. A field trial was sown in early November 2002 and harvested in early March 2003. A total of 39 lines that contained the CPR transgene in a CO48ib parent background were tested in the field. The morphine, thebaine, and total alkaloid content of these lines varied considerably. The transgenic line 8951 had a significant increase of 15% in morphine content and 22% in total alkaloid content when compared to parent controls. For this line and other lines that performed well, increases were seen primarily, but not solely, in morphine and thebaine content. Figure 12 is attached as Exhibit D and provides a comparison of field grown CO48ib transgenic lines and parent controls. The parent lines are prefixed by "P" and the tissue culture controls (non-transgenic plants which have been through tissue culture) are prefixed by "TC".

9. Comparisons of the genetically modified poppy lines and the commercial lines were made and the best CPR line outperformed the best commercial line by 4.5% for morphine content and 8.5% for total alkaloid content. This is a significant result because the GM poppies were produced from poorer parent backgrounds than the commercial varieties.

10. In summary, at least on the basis of the experimental results described herein, it is my opinion as a skilled practitioner that over-expression of cytochrome P-450 reductase in a poppy plant results in increased alkaloid content of the poppy straw of a poppy plant.

Date: 16 September 2004 By: A. J. F.
Anthony John Fist